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(54) Title: ASCORBATE–ISOQUERCETIN COMPOSITIONS

(57) Abstract

The present invention relates to novel compositions containing ascorbic acid and one or more derivates of quercetin which orally administred conveys in vivo higher protection, longer maintenance of biological activity, higher concentration in tissues and higher biological efficiency to vitamin C in organs in human body. These compositions are useful as pharmaceutical compositions and as food supplements possessing preventive properties against damages of human organs, including skin, tissues and cells due to oxidative stress or damages.

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Ascorbate-Isoquercetin Compositions

The present invention relates to novel compositions containing ascorbic acid with an increased level of its active form. These compositions are useful as food supplements possessing preventive properties against damages of human tissues, including skin cells due to oxidative stress.

In vivo ascorbic acid (vitamin C) exists in three forms:
a) as an ascorbate in form of an ascorbate monoanion,
b) as the reversibly oxidised form of a free radical, called semidehydroascorbic acid which could be reversibly oxidised to dehydroascorbic acid or reversibly reduced to ascorbate monoanion, and
c) as dehydroascorbic acid (oxidised form of semidehydroascorbic acid).

Only ascorbate possesses specific vitamin C activity: as a cofactor for enzymes. Observed physiological activities of semidehydroascorbic acid and dehydroascorbic acid formed in vivo from ascorbate are considered to be based on their reversible reductions to ascorbates, (Buettner, 1993- Dharival et al., 1991; Welch et al., 1995 Washko et al., 1993).The second form of ascorbic acid, semidehydroascorbic acid (ascorbate free radical) participates in univalent redox systems, (Bors et al. 1995), that is in the antioxidant defence activity. This means, semidehydroascorbic acid participates most likely in free radical scavenging activities. According to Gordon (1996, p. 270), "ascorbate appears to be the most important non-protein antioxidant in plasma". Ascorbic acid is absorbed from the gastrointestinal tract in the form of ascorbic acid. Dehydroascorbic acid is reduced to ascorbic acid for gastrointestinal absorptions (Rose et al., 1988).

Structures of body tissues are susceptible to damages caused by the oxidative stress, e.g. by the accumulation of reactive oxygen species during ageing, chronic environmental stress, inflammations or general metabolic dysfunctions. The role of free radicals and reactive oxygen species in aetiology of human diseases (e.g. cancer, atherosclerosis, rheumatoid

arthritis, inflammatory bowel diseases, immune system dysfunctions, brain function decline, connective tissue dysfunctions) is well established (for a recent review see: Gordon, 1996). Uncontrolled generation of free radicals, especially chronic exposure to reactive oxygen species leads to chronic intracellular damages, to oxidative stress and premature ageing. Cells of the human body possess metabolic antioxidant defences which are supported by dietary antioxidants. The early observations of the antioxidant defence metabolic processes involved vitamin C and flavonoids (Bezssonoff, 1926; Bentsath et al., 1936; Bensath et al., 1937; Blanc and Von der Muehl, 1967). Ascorbic acid is not only important non-protein antioxidant in human plasma (Gordon, l.c.) but it increases (Skaper et. al.,1997) the cytoprotective activities of quercetin and rutin. Skaper and co-authors (1997) have shown, for instance, that quercetin protects connective tissue and specifically skin cells (e.g. fibroblasts, keratinocytes, and endothelial cells) from this type of damages. Other authors have demonstrated protective effect of flavonols on cardiovascular and nervous system, their role as chemoprotective agents in carcinogenesis.

Oxidation of the ascorbate in the human body by xenobiotics often leads to the accumulation of semidehydroascorbic acid or dehydroascorbic acid in organs where these forms interfere with the regular metabolism. As ascorbate is a cofactor for eight isolated enzymes (carrying out collagen synthesis,carnitine synthesis, peptide amidation, tyrosine metabolism, and catecholamine synthesis) the decrease of the concentration of ascorbate in body tissues and fluids may leads to seroius metabolic dysfunctions.

The possibilities to protect ascorbic acid in vivo were based on very early observations of Szent-Györgyi group mentioned above that the ascorbic acid activity in humans and guinea pigs is intensified by the great group of "vegetable dyes, the flavons or flavonols". It has been known that flavonoids are contributing to the maintenance of the concentration of the administered ascorbate in adrenals, kidneys, spleen, and the liver of the organisms investigated and improve the antiscorbutic effect of the dosages of

ascorbate used (Papageorge and Mitchell, 1948; Cotereau et al., 1948; Crampton and Lloyd, 1950; Douglas and Kamp, 1959; Blanc and Von der Muehl, 1967; Zloch, 1973).

The mechanism of this effect, called "the vitamin C-economising function" of some flavonoids ("facteur d'economie de L'acide ascorbique" of Bezssonoff, 1926 and 1927) has been recognised in many laboratories. For example, Harper et al., 1969, found that, among flavonoids tested, flavonols the have strongest ability to inhibit ascorbic acid oxidation in near neutral solutions (pH 5 - 7) . Harper et al. (1.c.) also pointed out that the presence of free hydroxyl groups at carbon atoms 3,7, 3', and 4' in a flavonol molecule improves the antioxidative effect of the flavonol molecule, this means, it inhibits ascorbate oxidation more effectively.

But there was neither an effective method nor a useful orally applicable formulation leading to an increased level of active ascorbate in human tissue.

Accordingly, there was a need for a composition useful for the protection of the orally administered ascorbic acid and enhancement of vitamin activity in the tissues.

Now it has been found that isoquercetin effectively inhibits ascorbate oxidation. The maintenance of the reduced form of ascorbic acid by isoquercetin maintains ascorbic acid level in body tissues and fluids.

This effect perhaps may be explained in that isoquercetin not only shows three free hydroxyl groups mentioned by Harper (1.c.), more exactly, hydroxyl groups attached to carbon atoms 7, 3', and 4', but also a glucopyranoside moiety with additional four free hydroxyl groups 0-attached to the carbon 3 of isoquercetin. Therefore, the increased effectivity of ascorbate protectioin may be caused by the fact that isoquercetin contains a glucose molecule. This glucose molecule seems to be the reason why isoquercetin is able to use the sodium-dependent glucose transport

pathway of the intestinal brush-border membrane in its absorption process (Gee et al., 1998). Experiments have also shown that the absorption of isoquercetin is better than that of pure aglycone.

Earlier pharmacokinetic studies with isoquercetin anticipated results obtained and explained by Gee at al,. l.c., by having shown excellent absorption rate and bioavailability of isoquercetin (Hollman and Katan, 1997).

It has been found that ascorbate is not only able to regenerate oxidised flavonols by reducing them (Yamasaki et al., 1997) but also to protect quercetin (aglycone of the isoquercetin) against oxidative degradation and to maintain the antiviral properties of quercetins (Vrijsen et al., 1988).

This means, there is a synergistic effect between isoquercetin and ascorbate in human tissue leading to higher effectivities of both, ascorbate and isoquercetin.

For isoquercetin these activities are as follows:
it has shown anthypertensive properties, (Kameda et al., 1987); it inhibits the biosynthesis and release of prostaglandin-like substances (Chanh et al. 1986); it produces dose-dependent protection in oxidative DNA damage (Noroozi et al., 1998) , it possesses preventive properties against damages of vascular and connective tissues (especially skin) and it is therapeutically useful in the treatment of dysfunctions of the digestive tract (Seto et al. 1992).

Now we have found by experiments that the combination of vitamin C with the most easily bioavailable bioflavonoid, isoquercetin, is most effective in prevention of and in defense against stress dysfunctions, especially against oxidative damages of living tissues including brain, vascular, connective tissues (especially skin).

It has been found that a composition containing ascorbic acid and one or more derivates of quercetin elected from the group quercetin-3-0-glucoside (isoquercetin), quercetin-4'-glucoside, quercetin-3'-glucoside and acid-quercetin-7-glucoside in a molar ratio of ascorbate to flavonoid in the range of 2:1 to 1 : 2, preferably in the molar ratio of 1 : 1, orally administered conveys in vivo higher protection, longer maintenance of biological activity, higher concentration in tissues and higher biological efficiency to vitamin C in organs of human body. This adduct similarly also provides the properties of higher protection, longer maintenance of biological activity, higher concentration in tissues, and higher biological efficiency in organs of human body to isoquercetin and the other glucosides of the above mentioned group.

Useful compositions may contain in a daily dose 30 - 4000 mg of an active amount of ascorbic acid or preferably of physiologically active ascorbate in form of its sodium salt, calcium, other mineral, or organic cation salts. Usually compositions contain 150 - 1000 mg, but for special treatments the amount is chosen higher between 1000 and 4000 mg, preferably between 1500 and 3000 mg.The compositions according to the present invention may be prepared in form of tablets, capsules or syrups. These application forms may also contain further active ingredients in useful amounts like vitamins, suitable salts of Mg, Ca, K or Fe and perhaps trace elements.

The compositions of the present invention preferably are useful as food supplements, but they may also be administered in a pharmaceutical treatment.

The present invention makes available

a) a method of maintaining long biological activity and high concentration of ascorbate and isoquercetin in human organs (including skin), tissues and cells,

b) a method of protection against oxidative damages of human organs, tissues, skin cells,

c) a method of prevention of arteriosclerosis, cardiovascular diseases, and other damages of vascular tissues, of allergic and inflammatory disorders, of bacterial and viral infections, of metabolic dysfunctions involving oxidative damages e.g., premature ageing,

d) a method of supporting pharmacological treatments of diseases and dysfunctions caused by oxidative damages,

by orally administration of a composition described above. Generally speaking, compositions that are applicable contain at least ascorbic acid or ascorbate or any other form of this vitamin that would in vivo yield ascorbate, or semidehydroascorbic acid, or dehydroascorbic acid and isoquercetin. The decision which further ingredients should be components of a composition useful in one of the above mentioned methods depends on the special indication. Usually, if the composition is administered as a way of protection or prevention useful further ingredients may be further vitamins, salts of Mg, Ca, K, Fe and trace elements in known amounts as used in food supplements. Compositions useful in method of supporting pharmacological treatments may differ from them.

The superiority of isoquercetin and ascorbate used in combination for the protection of human cells, tissues and organs from the oxidative stress is based on two properties of isoquercetin and of ascorbate. First, on the quick intestinal absorption of orally administered isoquercetin and of ascorbate, and on the rapid and simple passage of both compounds through cytomembranes of human organs; secondly, on the specificity of interaction of isoquercetin with ascorbate. Specifically, ascorbate maintains isoquercetin in its active oxidised state and isoquercetin maintains ascorbate in its enzymatically active reduced state.

On the basis of our research on the bioavailability and on redox properties of isoquercetin and ascorbate it has been found that orally administered mixtures of isoquercetin and ascorbate are most effective in protecting the

organs (including skin), tissues, and cells from the chronic intracellular oxidative damages.

The uptake of isoquercetin into the human body is facilitated by the sodium-dependent glucose transport system. This type of transport occurring in most animal species (Coady et al., 1990) is active during the uptake of pyranosides as for example described by Hediger for methyl alpha-D-glucopyranoside (Hediger et al., 1987). The sodium-dependent glucose transport system in mammals was studied in many laboratories. Koepsell and Spangenberg (1994) characterised Na(+)-D-glucose cotransport in the intestine. It is a cotransporting system composed of a set of two subunits: transport-mediating proteins and transport-modulating proteins. The first translocates the substrates and the second accelerates the V_{max} of the Transport. The susceptibility of isoquercetin to be transported using the Na(+)-D-glucose cotransport is suggested to be determined by the manner in which a non-glucose moiety is linked to glucose. More information about this is given in a review of Olson and Pessin, 1996. Direct evidence that isoquercetine uses sodium-dependent glucose transport pathway of the intestinal brush-border membranes was obtained by Gee et al., 1998.

Also the uptake of ascorbate by human is caused by a sodium dependent glucose transport system. Interactions between glucose and ascorbate transport activity have been demonstrated in many tissues and cells (Rumsey and Levine, 1998). Apparently ascorbate is absorbed in human intestine by a sodium-dependent active transport system, although in vitro about 10-20% of ascorbic acid moves into cells in the absence of sodium (Kuo et al., 1997). The carrier proteins in the intestinal cell membranes bind and transport the vitamin across the membrane to its intracellular site of action. There are differences in transport kinetics, tissue specificity, Na^+-dependence and energy dependence (Rumsey and Levine, 1.c.), but in most cases the transport of ascorbate is Na^+-dependent and requires metabolic energy. Kinetic evidence suggests strongly that ascorbate may be

transported by the same transporter as glucose and, therefore, by the same transporter as isoquercetin.

Pharmacokinetic studies with isoquercetin support the present invention as they show excellent absorption rate and bioavailability of isoquercetin. It is absorbed better than rutin and quercetin (Hollman, 1997). Absorbed isoquercetin interacts with ascorbate protecting it and, at the same time, is being protected by ascorbate by being kept in the reduced state (Yamasaki et al., 1997). It has also been shown that ascorbate protects quercetin (aglycone of the isoquercetin) against oxidative degradation and maintains quercetin's antiviral properties (Vrijsen et al., 1988). Effectiveness of isoquercetin in interacting with ascorbate is strengthened by the fact that isoquercetin uses the preferential intestinal Na(+)-D-glucose cotransport discussed above.

Therefore, a most powerful dietary antioxidant composition is prepared using among other ingredients ascorbic acid and isoquercetin. The advantageous properties of these compositions are induced by the synergistic effect of Isoquercetin protecting the activity of the orally administered ascorbic acid while maintaining its enzymatically active reduced form, and, on the other side, of ascorbate maintaining isoquercetin in its active oxidised state.

Surprisingly it was found that in contrast to other quercetin glucosides, isoquercetin shows far better absorption rates in human intestinal tract than rutin or the quercetin aglycone and that it acts as a specific and most powerful dietary antioxidant at the same time.

This positive result was unexpected because mixtures of ascorbic acid and quercetin or quercetin glucosides other than isoquercetin were considerably less effective .

Subject of this invention is that in humans the oral administration of a mixture or combination of ascorbic acid and isoquercetin (quercetin-3-0-glucoside); or of any of mixtures of ascorbic acid and quercetin-4'-glucoside; of ascorbic acid and quercetin-3'-glucoside; of ascorbic acid and quercetin-7-glucoside, with a suitable molar ratio, preferably equimolar ratio, of ascorbate to flavonoid, conveys efficient protection against oxidative damages, due to long maintenance of biological activity of each of the ingredients and due to maintenance of high concentration of both ascorbate and isoquercetin in organs, tissues, and cells.

The invention of this application includes especially compositions containing the above mentioned ingredients useful for the prevention and treatment of atherosclerosis and other cardiovascular disorders, certain forms of cancer, allergic and inflammatory disorders, bacterial and viral infections, a number of metabolic dysfunctions, e.g. premature ageing and other pathological conditions that involve oxidative damages.

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CLAIMS

1. Orally applicable composition containing ascorbic acid or ascorbate or its derivative in combination with one or more derivatives of quercetin elected from the group quercetin-3-O-glucoside (isoquercetin), quercetin-4'-glucoside, quercetin-3'-glucoside, and quercetin-7-glucoside.

2. Composition according to claim 1 containing isoquercetin in combination with ascorbic acid or of a physiologically active ascorbate in form of of its sodium, calcium, other mineral or organic salts.

3. Composition according to claims 1 or 2 containing a combination of isoquercetin and ascorbic acid or their mineral or organic salts and additionally other ingredients.

4. Composition according to claim 3 wherein other ingredients are vitamins.

5. Composition according to claims 1 - 4 wherin other ingredients are suitable salts of Mg, Ca, K, and Fe.

6. Composition according to claims 1 - 5 wherin other ingredients are trace elements.

7. Composition according to claims 1 - 6 containing ascorbic acid or ascorbate and isoquercetin in a molar ratio in the range of 2 : 1 to 1 : 2.

8. Composition according to claims 1 - 6 containing ascorbic acid or ascorbate and isoquercetin in a molar ratio in the range of 1 : 1.

9. Compositions according to claims 1 - 8 containing 30 - 4000 mg ascorbic acid or ascorbate in daily dose, preferably 150 - 1000 mg.

10. Compositions according to claims 1 - 8 containing 1500 - 3000 mg ascorbic acid or ascorbate in daily dose.

11. Use of compositions according to claims 1 - 10 as a food supplement.

12. Method of maintaining long biological activity and high concentration of ascorbate and isoquercetin in human organs, especially skin, tissues and cells by orally administration of a composition according to claims 1 - 10.

13. Method of protection against oxidative damages of organs, including skin, tissues and cells by orally administration of a composition according to claims 1 - 10.

14. Method of prevention of arteriosclerosis, cardiovascular diseases, allergic and inflammatory disorders, bacterial and viral infections, metabolic dysfunctions, e.g. premature ageing, and of other pathologic conditions involving oxidative damages by orally administration of compositions according to claims 1 - 10.

15. Method of supporting pharmacological treatments of diseases and dysfunctions caused by oxidative damages by orally administration of compositions according to claims 1 - 10.

16. Pharmaceutical composition containing a compositions according to claims 1 - 9.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
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International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/06166	23/08/1999	27/08/1998
International Patent Classification (IPC) or national classification and IPC A61K31/35		
Applicant MERCK PATENT GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07/02/2000	05.12.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Heller, D Telephone No. +49 89 2399 8746

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/06166

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-12 as originally filed

Claims, No.:

1-16 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
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3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 12-15.

because:

☒ the said international application, or the said claims Nos. 12-15 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7
	No:	Claims	1-6,8-16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-16
Industrial applicability (IA)	Yes:	Claims	see sections III and V

No: Claims

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION III:

Claims 12 to 15 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

SECTION V:

Prior art

Reference is made to the following documents:

D1 (PATENT ABSTRACTS OF JAPAN vol. 016, no. 338 (C-0965), 22 July 1992 (1992-07-22) & JP 04 099771 A) is directed to a drug, cosmetic or food, etc., in which ascorbic acid or a derivative of said compound is used is mixed with flavonoid glycoside in a same or less amount as ascorbic acid. The flavonoid glycoside is a species or a mixture of two or more species of rutin, quercetin, isoquercetin, pertatoside and hyperoside, or a glycoside readily soluble in water (abstract).

D2 (DATABASE WPI Section Ch, Week 199433 Derwent Publications Ltd., London, GB; Class B05, AN 1994-269369 XP002123121 & JP 06 199693 A) relates to an agent comprising a substance having superoxide dismutase (SOD) activity and/or antioxidative activity (including scavenger activity), a phenol compound and sugar compound such as glycoprotein and saccharified flavonoid (abstract).

D3 (DATABASE WPI Section Ch, Week 199433 Derwent Publications Ltd., London, GB; Class B05, AN 1994-269367 XP002123122 & JP 06 199690 A) is directed to the same compounds as D2 for activating the cerebral metabolism to improve the memory and cerebral function, free from side action and useful for the treatment of cerebral and neurologic diseases (abstract).

D4 (DATABASE WPI Section Ch, Week 199715 Derwent Publications Ltd., London, GB; Class A96, AN 1997-161434 XP002123119 & JP 09 030987 A) relates to a

preparation for treating and preventing an intractable ulcer, gastritis and dermatitis resulting from a pathogenic microorganism as a cause, by selecting antimicrobial inhibiting substances and combining them. Preventive and treating agent comprises (i) antioxidative natural product or synthetic compound; (ii) antioxidative antibacterial substance; and/or iii) basic polysaccharide. (abstract).

D5 (DATABASE WPI Section Ch, Week 199539 Derwent Publications Ltd., London, GB; Class B02, AN 1995-299503 XP002123120 & JP 07 196523 A) describes a solution for internal use capable of supplying metallic ions deficient in a human body, simultaneously promoting metabolic functions in the liver, thereby maintaining the body healthy, further recovering the fatigue, adjusting the physical condition and imparting vitality. This solution for internal use contains (A) a glycoside of quercetin, preferably a glycoside of a solubilized quercetin included with a water-soluble rutin or cyclodextrin, (B) a bivalent metallic ion, i.e., at least one of calcium, magnesium or zinc ions and (C) an extract of Glycyrrhizae Radix containing glycyrrhizin (abstract).

D7 (Noroozi M. et al.; cited in the application) assesses the antioxidant potencies of several dietary flavonoids across a range of concentration and compared with vitamin C as a positive control in vitro (abstract).

D8 (Vrijsen R.et al.; cited in the application) discloses the in vitro use of ascorbate and quercetin in combination having antiviral activity (p. 1749, §3).

D9 (Seto T. et al.; cited in the application) discloses the extraction and isolation of several flavonoid components from Pseudocarps or seeds of R.multiflora (abstract).

Novelty

The subject-matter of claims 1 to 6, 8 to 16 is not new in the sense of Article 33 (2) PCT.

The following claims are not new over the following documents with their cited passages:

Claim 1: D1: abstract

	D2:	abstract
	D3:	abstract
	D4:	abstract
	D5:	abstract
	D8:	p. 1749, §3, Fig. 3
Claim 2:	D1:	abstract
	D2:	abstract
	D3:	abstract
Claim 3:	D2:	abstract
	D3:	abstract
Claim 4:	D2:	abstract
	D3:	abstract
Claim 5:	D2:	abstract
	D3:	abstract
	D5:	abstract
Claim 6:	D2:	abstract
	D3:	abstract
	D5:	abstract
Claim 8:	D1:	abstract
Claim 9:	D2:	abstract
	D3:	abstract
	D4:	abstract
Claim 10:	D2:	abstract
	D3:	abstract
	D4:	abstract
Claim 11:	D1:	abstract
	D2:	abstract
	D3:	abstract
	D4:	abstract
	D5:	abstract
Claim 12:	D2:	abstract
	D3:	abstract
	D4:	abstract
	D5:	abstract
Claim 13:	D2:	abstract

	D3:	abstract
	D4:	abstract
	D5:	abstract
Claim 14:	D2:	abstract
	D3:	abstract
	D4:	abstract
	D5:	abstract
Claim 15:	D2:	abstract
	D3:	abstract
	D4:	abstract
	D5:	abstract
Claim 16:	D1:	abstract
	D2:	abstract
	D3:	abstract
	D4:	abstract
	D5:	abstract
	D8:	p. 1749, §3, Fig. 3

As D7 discloses comparative in vitro experiments which are not directed to a combination of vitamin C and flavonoids, D7 does not anticipate novelty of present claims 1 to 16.

D9 gives only an overview over the extraction and isolation of several flavonoids.

Inventive step

The subject-matter of claims 1 to 16 does not involve an inventive step in the sense of Article 33 (3) PCT.

For claims 1 to 6 and 8 to 16 the following applies:
Even if the applicant is able to establish novelty it cannot be seen that any particular aspect of the application as filed would involve an inventive step under Article 33 (3) PCT in the light of the relevant prior art.

For claim 7 the following applies:

The additional features of claim 7 can be determined by routine experiments and are therefore not inventive in the sense of Article 33 (3) PCT.

Industrial applicability

For the assessment of the present claims 12 to 15 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

SECTION VI:

The applicant is informed that no check has been made as to whether priority has been validly claimed. Therefore, document D6 (DE 198 20 680 C), which has been disregarded in writing the present opinion, could become relevant for the assessment of novelty once the present application enters the regional phase (Rule 64 (1) b PCT).

SECTION VIII:

Claims 5 and 6 are not clear according to Article 6 PCT, because they are dependent to claims 1 to 4/5. They could only be dependent to claim 3, because claims 1 and 2 are silent to "other ingredients".

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing: 09 March 2000 (09.03.00)	
International application No.: PCT/EP99/06166	Applicant's or agent's file reference: 0098326-Wguc
International filing date: 23 August 1999 (23.08.99)	Priority date: 27 August 1998 (27.08.98)
Applicant: BUCHHOLZ, Herwig et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

07 February 2000 (07.02.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

MAH

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
0098326-Wguc		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/06166	23/08/1999	27/08/1998
Applicant		
MERCK PATENT GMBH et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[ ] the text is approved as submitted by the applicant.

[X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

The present invention relates to novel compositions containing ascorbic acid and one or more derivates of quercetin which orally administred conveys in vivo higher protection, longer maintenance of biological activity, higher concentration in tissues and higher biological efficiency to vitamin C in organs in human body. These compositions are useful as pharmaceutical compositions and as food supplements possesing preventive properties against damages of human organs, including skin, tissues and cells due to oxidatie stress or damages.

INTERNATIONAL SEARCH REPORT

International Application No
T/EP 99/06166

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/35 A61K31/375 A23L1/302 A23L1/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 016, no. 338 (C-0965), 22 July 1992 (1992-07-22) & JP 04 099771 A (SAN EI CHEM IND LTD), 31 March 1992 (1992-03-31) abstract ---	1
X	DATABASE WPI Section Ch, Week 199433 Derwent Publications Ltd., London, GB; Class B05, AN 1994-269369 XP002123121 & JP 06 199693 A (KATO K), 19 July 1994 (1994-07-19) abstract --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 November 1999	14/12/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Caturla Vicente, V

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
E	DE 198 20 680 C (ECKES-GRANINI GMBH & CO.) 16 September 1999 (1999-09-16) page 3, line 49-67; claims 1,3	1,11
X	DATABASE WPI Section Ch, Week 199433 Derwent Publications Ltd., London, GB; Class B05, AN 1994-269367 XP002123122 & JP 06 199690 A (KATO K), 19 July 1994 (1994-07-19) abstract	1
A	DATABASE WPI Section Ch, Week 199715 Derwent Publications Ltd., London, GB; Class A96, AN 1997-161434 XP002123119 & JP 09 030987 A (ITO M), 4 February 1997 (1997-02-04) abstract	1,12-16
A	DATABASE WPI Section Ch, Week 199539 Derwent Publications Ltd., London, GB; Class B02, AN 1995-299503 XP002123120 & JP 07 196523 A (ITO M), 1 August 1995 (1995-08-01) abstract	
A	NOROOZI M. ; ET AL: "Effects of Flavonoids and Vitamin C on oxidative DNA damage to Human Lymphocytes" AMERICAN JOURNAL OF CLINICAL NUTRITION, vol. 67, 1998, pages 1210-1218, XP002124008 cited in the application	
A	VRIJSEN R; ET AL: "Antiviral Activity of Flavones and Potentation by Ascorbate" JOURNAL OF GENERAL VIROLOGY, vol. 69, 1988, pages 1749-1751, XP002124009 cited in the application	
A	SETO T; ET AL: "Purgative Activity and Pricipals of the Fruits of Rosa multiflora and R. wichuraiana" CHEM. PHARM. BULL., vol. 40, no. 8, 1992, pages 2080-2082, XP000857097 cited in the application	

3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/06166

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 04099771 A	31-03-1992	NONE	
JP 6199693 A	19-07-1994	NONE	
DE 19820680 C	16-09-1999	EP 0954986 A	10-11-1999
JP 6199690 A	19-07-1994	NONE	
JP 9030987 A	04-02-1997	NONE	
JP 7196523 A	01-08-1995	NONE	